capping material (e.g., wax). The device may be then packaged together with a cooling element (e.g., ice, dry ice, a thermoelectric chiller, etc.) and all may be placed in a (preferably insulated) package.

[0076] In some embodiments, to store and transport the device, a transient protective support media that is a flowable liquid at cooled temperature (e.g., 4° C.), but gels or solidifies at warm temperatures such as room temperature (e.g., 20° C.) or body temperature (e.g., 37° C.), such as poly(N-isopropylacrylamide) and poly(ethylene glycol) block co-polymers, may be added into the device to substantially or completely fill the chamber(s), and preferably also any associated conduits.

[0077] Upon receipt, the end user may simply remove the device from the associated package and cooling element, may allow the temperature to rise or fall (depending on the choice of transient protective support media), may uncap any ports, and may remove the transient protective support media with a syringe (e.g., by flushing with growth media).

[0078] The present invention is explained in greater detail in the following non-limiting Examples.

EXAMPLES

[0079] An airway organoid was constructed using primary normal human bronchial epithelial (NHBE) cells, primary human lung fibroblasts and human endothelial cells (HUVEC), as found in the normal human upper airway, layered on a polyester membrane in an order and ratio to replicate human airway tissue.

1. Construction of Lung Organoid.

[0080] Each side of a polyester membrane (Corning® Costar® Snapwell™ cell culture inserts, 12 mm with 0.4 μm pore, pore density 4×106 pores/cm^2, polyester membrane, TC-treated, sterile) was coated with 150 μl collagen IV (Sigma C7521) and left under the biosafety hood overnight (hood open and blower on). The membrane was UV sterilized for 30 mins the next morning.

[0081] 250,000 HUVECs (endothelial cells) (Cell and Viral Vector Core Laboratory, Wake Forest University) were seeded on the underside of the membrane and let stand for up to 4 hours for cells to attach. Membrane was then placed with 2 ml of EGM-10 in the well of the 6 well plate.

[0082] 250,000 HALF (human adult lung fibroblasts, isolated from donor) were seeded on the upper side of the membrane and covered with 200 μl of DMEM-F12 1:1 (Hyclone SH30261.01).

[0083] After 5-7 days, a layer of 250,000 cells NHBE cells (epithelial cells) (Lonza Cat # CC2540) was established over the layer of fibroblasts and covered with 200 μl BEGM (Clonetics CC4175).

[0084] Change media: EGM 10 and BEGM—Once every 2 days; DMEM—Once every 3-4 days by carefully pipetting off 100 μl media from the top of the transwell (for HALF& NHBE) without touching the cells and replacing it with the same amount of fresh media.

[0085] Primary cell types were characterized by PCR and flow cytometry to demonstrate normal cell phenotype using Vybrant® Multicolor Cell-Labeling Kit V-22889 (Used for labeling cells for imaging). p63-α (D2K8X) XP® Rabbit mAb #13109, Anti-Dynein intermediate chain 1 antibody [74.1] (ab23905) and Anti-Mucin 5AC antibody [45M1] (ab3649) were used for characterization of NHBE by flow-cytometry.

[0086] A microfluidic device was used to provide physiological flow of media to both sides of the membrane, or alternatively, to the lower side only, producing an air-liquid interface established at the upper membrane.

[0087] Pathogenesis of *Bordetella pertussis* was studied in this model by analyzing trans-epithelial resistance, the levels of toxins and cytokines, imaging by fluorescent microscopy.

2. Results and Discussion.

[0088] Characterization of cells: Primary lung fibroblasts maintained expression of fibroblast-specific markers during passaging, while NHBE cells maintained expression of markers for basal, goblet, ciliated and clara cells during culture.

[0089] Normal human lung fibroblasts are tested for expression of von Willebrand factor/Factor VIII, cytokeratins 18 and 19, and alpha smooth muscle actin. Lung microvascular endothelial cells express CD31/105, von Willebrand Factor, and PECAM. Human Primary lung Epithelial cells express markers basal (p63+ KRT5+), ciliated (Foxj1+ Sox17+), Clara (ScgblA1+) mucosal (Muc5ac+) cell numbers and CFTR+ epithelial cells.

[0090] Organoid Development: Fluorescent labeling of the cells of the airway organoid demonstrated that the cells formed distinct cell layers representative of endothelial vasculature, the stromal component and a polarized epithelial monolayer. TEM and histological imaging confirmed the development of a multi-layered upper airway organoid construct.

[0091] Maintenance of cells in microfluidic system: The airway organoid maintained in the microfluidic system remained viable and facilitated non-invasive analysis of *Bordetella pertussis* pathogenesis.

CONCLUSION

[0092] Multiple primary airway cell types were combined to generate a functional upper airway organoid. This bioengineered organoid system is useful in conducting studies into human disease, toxicity studies and drug and vaccine development.

[0093] The foregoing is illustrative of the present invention, and is not to be construed as limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein.

That which is claimed is:

1. A method of making a lung organoid, the method comprising:

depositing an endothelial cell layer comprising a mammalian endothelial cells onto a first side of a porous membrane;

depositing a stromal cell layer comprising a mammalian lung fibroblast cells onto a second side of the porous membrane that is opposite the first side of the porous membrane; and

depositing an epithelial cell layer comprising a mammalian lung epithelial cells directly onto the stromal cell layer.

2. The method of claim **1**, wherein said depositing steps are carried out by spreading, painting, coating, printing, bioprinting, and/or spraying.